

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:	)	Group Art Unit: 1635
BECKER <i>et al.</i>	)	Examiner: Shibuya, M.
Serial No. 09/523,237	)	Atty. Docket No. GP068-03.CN1
Filed: March 10, 2000	)	
For: KITS FOR AMPLIFYING TARGET	)	
NUCLEIC ACID SEQUENCES USING	)	
MODIFIED OLIGONUCLEOTIDES	)	

**DECLARATION UNDER 37 C.F.R. § 1.131**

Box Non-Fec Amendment  
Commissioner for Patents  
Washington, D.C. 20231

Sir:

We, Michael M. Becker, Steven T. Brentano and Mehrdad Majlessi, co-inventors of the above-identified patent application, hereby declare as follows:

1. Prior to August 25, 1995, we conceived of and reduced to practice in the United States modified oligonucleotide primers for use in amplifying target nucleic acid sequences, where the modified oligonucleotide primers contained one or more ribonucleotides having a 2'-O-methyl substitution to the ribofuranosyl moiety. Evidence of this prior conception and reduction to practice can be found in attached Exhibit A, which comprises a set of Steven Brentano's laboratory notebook pages setting forth a study which was conducted to test the efficacy of primers containing 2'-O-methyl substitutions in a transcription-mediated amplification procedure. Although the dates on these pages have been redacted, the study set forth therein was completed in the United States prior to August 25, 1995.

2. The amplification study set forth in Exhibit A included primer sets of both T7 and non-T7 oligonucleotide primers. The T7 primers of this study were 50 bases in length,

Considered KAL 04-12-02

## DECLARATION

Serial No. 08/893,300  
Atty. Docket No. GP068-02.UT

possessed the same base sequence (taking into account DNA/RNA equivalents), and differed in their structures only as follows: (i) the "T7ArpoHIV4195(-)" primers contained only unmodified deoxyribonucleotides; (ii) the "T7ArpoHIV4195(-)m13" primers contained 37 unmodified deoxyribonucleotides and 13 2'-O-methyl modified ribonucleotides positioned at the 3' most end of these primers; (iii) the "T7ArpoHIV4195(-)m18" primers contained 32 unmodified deoxyribonucleotides and 18 2'-O-methyl modified ribonucleotides positioned at the 3' most end of these primers; (iv) the "T7ArpoHIV4195(-)r13" primers contained 37 deoxyribonucleotides and 13 unmodified ribonucleotides positioned at the 3' most end of these primers; and (v) the "T7ArpoHIV4195(-)r18" primer contained 32 unmodified deoxyribonucleotides with the 18 unmodified ribonucleotides positioned at the 3' most end of these primers. A single non-T7 primer was used in this study, which is identified as the "HIV4116" non-T7 primer.

3. The primers of this study were all tested under essentially identical amplification conditions and at concentrations of 8, 15 or 30 pmol of the T7 primer and 30 pmol of the non-T7 primer in the presence of  $5 \times 10^5$  copies of an HIV target sequence or in the absence of the HIV target sequence. Following the addition of each primer set to an amplification reaction mixture under amplification conditions and for a period of time sufficient to amplify target sequence present in an amplification reaction mixture, a  $1 \mu\text{l}$  aliquot of amplification reaction mixture was removed from each  $100 \mu\text{l}$  amplification reaction mixture present in each reaction vessel. The  $1 \mu\text{l}$  aliquots of amplification reaction mixture were then added to separate vessels, each containing  $100 \mu\text{l}$  of deionized water.

4. Amplified target sequence present in the sample of each reaction vessel (either the remaining  $99 \mu\text{l}$  of undiluted amplification reaction mixture or the  $101 \mu\text{l}$  of diluted amplification reaction mixture) was then determined using a homogenous format described as the Hybridization Protection Assay (HPA) in the instant application, (see specification at page 5, lines 10-18), and acridinium ester (AE)-labeled probes specific for a target sequence present in the

## DECLARATION

Serial No. 08/893,300  
Atty. Docket No. GP068-02.UT

amplified HIV target sequence. Each sample received 0.1 pmol of AE-labeled probe and 4 pmol of identical cold probe, creating a competition assay as described in the instant application (*see* specification at page 10, lines 19-24). Signal from each sample was measured in relative light units (RLUs) using a luminometer.

5. The results of this study are recorded on page 67 of Exhibit A and are separated into various groupings based on the concentration and structure of the T7 primer tested. Those groups based on the concentration of target sequence present in the amplification mixture are identified as follows: (i) "-" represents the absence of target sequence in the amplification reaction mixture; (ii) "500 copies full format" represents the presence of  $5 \times 10^5$  copies of the HIV target sequence in the amplification reaction mixture prior to amplification and without any subsequent dilution; and (iii) "500 copies 1  $\mu$ l" represents the presence of  $5 \times 10^5$  copies of the HIV target sequence in the amplification reaction mixture prior to amplification, with 1  $\mu$ l of the amplification reaction mixture being diluted with 100  $\mu$ l of deionized water subsequent to amplification and prior to detection. And designations for the T7 primer structures are presented as follows: (i) fully deoxyribonucleotide T7 primers are designated as "N-8", "N-15" and "N-30"; (ii) T7 primers having 13 3' end 2'-O-methyl modified ribonucleotides are designated as "m13-8", "m13-15" and "m13-30"; (iii) T7 primers having 18 3' end 2'-O-methyl modified ribonucleotides are designated as "m18-8", "m18-15" and "m18-30"; (iv) unmodified T7 primers having 13 3' end ribonucleotides, with the remaining bases being deoxyribonucleotides are designated as "r13-8", "r13-15" and "r13-30"; and (v) unmodified T7 primers having 18 3' end ribonucleotides, with the remaining bases being deoxyribonucleotides are designated as "r18-8", "r18-15" and "r18-30". The second number in each case indicates the amount of T7 primer added to the amplification reaction mixture in pmol. All results are presented in terms of (RLUs) detected.

## DECLARATION


Serial No. 08/893,300  
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6. As the results of this study demonstrate, 2'-O-methyl modified primers can be used to successfully amplify target nucleic acid sequences. This is evidenced, for example, by a comparison of results from samples containing no target sequence with samples including either 2'-O-methyl modified primers or unmodified deoxyribonucleotide primers. (It is noted that the excessive RLU value for the "r18-15" sample under the "-" category on page 67 of Exhibit A would suggest that this sample was contaminated with target sequence.) The results for both the 13 and 18 base modified primers in these tests were very similar.

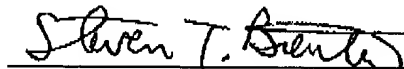
We hereby declare that all statements made herein of our own knowledge are true, and that statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of this application and any patent issuing therefrom.

Date: February 4, 2002

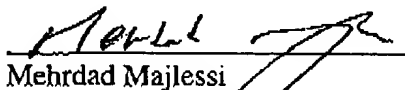
By:

  
Michael M. Becker, Ph.D.Date: 2-4-02

By:

  
Steven T. Brentano, Ph.D.Date: 2/4/02

By:

  
Mehrdad Majlessi

FEB- 6-02 WED 12:17 PM GEN-PROBE

FAX NO. 858 410 8928

P. 14/18

**EXHIBIT A**

TITLE 20M & RNA T7proHIV 4195E Primer Test Project No. AMP-T  
Book No. 3222

64

From Page No. X

Purpose: Test ~~the~~ <sup>new</sup> T7proHIV 4195E primer w/ either 13 or 18 bases of 20M or RNA on 3' end (see 3222:35283751).  
Use lots of target to start. Compare to normal 4195 primer. Check over all signal & consistency.

Procedure:

- amp w/ all three primers at 8, 15, or 30  $\mu$ l
- use HIV 4116 as non T7 at 30  $\mu$ l

	no.	oo/ $\mu$ l	ng/ $\mu$ l	Prod/ $\mu$ l
<del>HEB 4116</del>	mm 3184-33	47.8 <sub>500</sub>	0.712 <sub>100</sub>	43.4 36.4
T7AproHIV 4195E M13	mm 3189-42	19.8 <sub>100</sub>	0.712	43.4
T7AproHIV 4195E M18	mm 3189-42	19.75	0.712	48.2
T7AproHIV 4195E M13	mm 3227-8	6.5	0.26	15.86
T7AproHIV 4195E M18	mm 3227-6	9.9	0.396	24.16

all 50  $\mu$ l long

- non T7 =

~~HEB 4116~~ mm 3097:99 60.1  $\mu$ l prod/ $\mu$ l (35  $\mu$ l HEB aliquot)

- 3 @ & 3 @ amp ea primer, so make mix x 7

- target: use 500 copies ea amp (6  $\mu$ l min x 2 amp x 3  $\mu$ l = 45  $\mu$ l amp x 60)

- for 60 of 50  $\mu$ l x 500 copies =  $3 \times 10^8$  copies in 3  $\mu$ l

- target =  $2 \times 10^8$  copies/ $\mu$ l  
=  $2 \times 10^4$  / $\mu$ l

→ so use 1.5  $\mu$ l + 3  $\mu$ l H<sub>2</sub>O = 500 copies/50  $\mu$ l

no!  $2 \times 10^8$  / $\mu$ l  
so used 500,000 copies!

To Page No. 65

Witnessed &amp; Understood by me,

MHB

Date

Invented by

Steve Benth

Recorded by

Date

Project No. AMP-TBook No. 3222TITLE cont

65

From Page No 64

- make premix x165 (10500) at 25/1000

2875  $\mu$ l AR (2500061X recomb H<sub>2</sub>O)57.4  $\mu$ l H<sub>2</sub>O (210  $\mu$ l for 7 arpo = 3.49  $\mu$ l ea)

- amp mix x7

x7	77 $\mu$ l x1	77 $\mu$ l x7	77 $\mu$ l x7	premix
W-8	8 $\mu$ l	56 $\mu$ l	1.52 $\mu$ l	125 $\mu$ l
U-15	15	105	2.75	
U-30	30	210	5.49	
M13-8	8	56	1.29	
M13-15	15	105	2.72	
M13-30	30	210	4.74	
M18-8	8	56	1.16	
M18-15	15	105	2.18	
M18-30	30	210	4.36	
R10-8	8	56	3.53	
R13-15	15	105	6.62	
R13-30	30	210	13.24	
R18-8	8	56	2.32	
R18-15	15	105	4.35	
R18-30	30	210	8.69	

In the  
primer pool

- Do amp as usual by phage

general  
 MTD AR 9506061A  
 MTD ER 95030294  
 MTD EDB 9505032X

- 25  $\mu$ l amp mix / tube (3 @ 30 ea)- 50  $\mu$ l target in H<sub>2</sub>O

- 60°C 10 min, 92°C 5 min

- 42°C Add 25  $\mu$ l each rpt (2000u RT, T1)

- 42°C 90 min

To Page No 66

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Date

Invented by

Date

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TITLE contProject No. AMP-T  
Book No. 3222

66

From Page No. 65

- HPA 1  $\mu$ l  $\pm$  9.9  $\mu$ l } w/ 4  $\mu$ l cold crude  
+100  $\mu$ l H<sub>2</sub>O } 0.1  $\mu$ l AB

- probe mix x 160

16  $\mu$ l 2x high

16  $\mu$ l AB probe = 200  $\mu$ l x 80  $\mu$ l off  $\mu$ l

640  $\mu$ l cold crude = 1.5  $\mu$ l (at 42  $\mu$ l/ $\mu$ l)

NO! conc of  
crude = really  
5.45  $\mu$ l/ $\mu$ l  
so really 5  $\mu$ l/ $\mu$ l

- high 15 min 60°C

- 300  $\mu$ l out, DH 10 min 60°C

- H<sub>2</sub>O to cool

- read CFTD 2 sec

### Results:

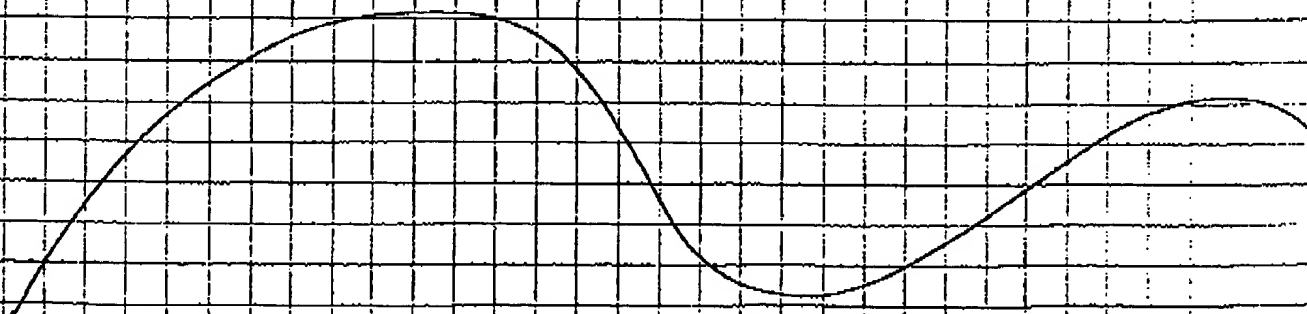
- data next page

-  $\odot$  all pretty high, ~10,000 RCU

- to bleach pipets for next time

★ all Normal c RNA c 2' OADe primer amp worked  
pretty well c had saturated signal even but only

- try again w/ less target

To Page No. 67

Witnessed &amp; Understood by me,

*[Signature]*

Date

Invented by

*[Signature]*

Date

Recorded by

*[Signature]*



Project No. AMP-TBook No. 3222TITLE Cont

67

From Page No. \_\_\_\_\_

PROTOCOL 18 LISTING

NAME: AMP-T  
 TYPE: AMP-T  
 DELAY BETWEEN INJECTIONS: 1 SEC  
 DELAY LAST INJECT TO COUNT: 2 SEC  
 COUNT TIME: 0.000  
 BLANK TIME DURATION: 2 SEC  
 NO. OF BLANK RUNS REQUIRED: 0  
 NO. OF SAMPLE REPLICATES: 3

PROTOCOL 18 RAW DATA

SOFTWARE REVISION: 1.00  
 PLOT SERIAL NUMBER: 12142

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 DELAY LAST INJECT TO COUNT: 2 SEC

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SAMPLE 2  
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 3 18628  
 CV= 5.8% AVG 18628

SAMPLE 3  
 1 16280  
 2 16280  
 3 16280  
 CV= 7.2% AVG 16280

SAMPLE 4  
 1 5620  
 2 5620  
 3 5620  
 CV= 1.1% AVG 5620

SAMPLE 5  
 1 6719  
 2 7125  
 3 7125  
 CV= 2.3% AVG 7125

SAMPLE 6  
 1 11070  
 2 11070  
 3 11070  
 CV= 5.0% AVG 11070

SAMPLE 7  
 1 7216  
 2 7216  
 3 7216  
 CV= 4.0% AVG 7216

SAMPLE 8  
 1 8748  
 2 8748  
 3 8748  
 CV= 7.0% AVG 8748

SAMPLE 9  
 1 11946  
 2 12218  
 3 12218  
 CV= 5.2% AVG 12218

SAMPLE 10  
 1 6887  
 2 6887  
 3 6887  
 CV= 2.7% AVG 6887

SAMPLE 11  
 1 9174  
 2 9174  
 3 9174  
 CV= 5.2% AVG 9174

SAMPLE 12  
 1 12095  
 2 11594  
 3 12218  
 CV= 5.2% AVG 11946

SAMPLE 13  
 1 8489  
 2 8489  
 3 8489  
 CV= 6.9% AVG 8489

SAMPLE 14  
 1 271491  
 2 271491  
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 CV= 1.4% AVG 271491

SAMPLE 15  
 1 8977  
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 CV= 6.1% AVG 8977

SAMPLE 16  
 1 265897  
 2 265897  
 3 265897  
 CV= 1.0% AVG 265897

SAMPLE 17  
 1 264231  
 2 264231  
 3 264231  
 CV= 1.2% AVG 264231

SAMPLE 18  
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SAMPLE 19  
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SAMPLE 20  
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SAMPLE 21  
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SAMPLE 22  
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SAMPLE 49  
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PROTOCOL 18 RAW DATA

SOFTWARE REVISION: 1.00  
 PLOT SERIAL NUMBER: 12142

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 DELAY LAST INJECT TO COUNT: 2 SEC

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 CV= 1.2% AVG 2404109

SAMPLE 18  
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 3 2404109  
 CV= 1.2% AVG 2404109

Witnessed & Understood by me, W. J. B. B.

Date

Invented by W. J. B. B.

Date

Recorded by W. J. B. B.To Page No. X